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New Mexico Environment Department (NMED) Surface Water Quality Bureau (SWQB)

Standard Operating Procedure (SOP) for

BACTERIOLOGICAL SAMPLING AND ANALYSIS

Approval Signatures

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Date

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1.0 Purpose and Scope

This procedure describes the collection and analysis of ambient water and wastewater samples for total coliform and *Escherichia coli* (*E. coli*) bacteria using the IDEXX Laboratories, Inc. Colilert® method for water quality standards assessment, complaint/spill response, discharge monitoring purposes, and the sampling for fingerprinting of microbial sources through laboratory analysis, also known as Microbial Source Tracking (MST). Sampling procedures for permit compliance monitoring are covered in SOP 8.3 – NPDES Wastewater Sampling (NMED/SWQB 2014 or most current).

2.0 Personnel Responsibilities

The Monitoring, Assessment, and Standards Section (MASS) Program Manager coordinates with the Subject Matter Expert (SME) and the Quality Assurance Officer (QAO) as applicable to ensure the procedures identified in this Standard Operating Procedure (SOP) for data collection, verification and validation are up-to-date and accurate so that the data results support program commitments.

The MASS Program Manager will provide input on the scope and intent of the SOP as it pertains to the program’s goals and objectives. The Program Manager will also review SOP every two (2) years after revision by Subject Matter Expert (SME) and/or QAO.

The QAO is involved in the development and revision of this SOP to ensure the SOP meets the requirements of the SWQB’s Quality Assurance Project Plan (NMED/SWQB 2021 or most current). The QAO, along with the SME and MASS Program Manager will determine if any revisions to this SOP are needed at a minimum of every two (2) years in accordance with the current version of the SWQB SOP 1.1 Creation and Maintenance of SOPs. Pending the review and approval of the document, the QAO will ensure the SOP is accessible through the SWQB’s website.

The SME reviews SOP every two (2) years and updates the SOP as the procedure or equipment changes in coordination with the QAO and MASS Program Manager.

All SWQB personnel who collect, process or analyze bacteriological samples are responsible for implementing procedures detailed in this SOP.

3.0 Background and Precautions

3.1 Background

The SWQB and the New Mexico Scientific Laboratory Division (SLD) both use the IDEXX Laboratories, Inc. Colilert® procedures for enumeration of total coliform and *E. coli* by the most probable number (MPN) method. The procedure is explained in the Colilert® reagent snap packs and in *Standard Methods*, Part 9000 (APHA 2017). Background on the MPN method can be found in Oblinger and J. A. Koburger (1975).

Microbial Source Tracking involves laboratory methods to identify the sources of fecal contamination through an analysis of genetic markers for host gut bacteria.

3.2 Procedural Precautions

E. coli is a pathogen and can be an indicator of pathogens harmful to humans and precautions should be taken when sampling potentially contaminated water. Avoid accidental ingestion, contact with mucous

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membranes, eyes and skin to the extent possible, especially areas with cuts and abrasions. Hepatitis vaccinations are available for staff, goggles and disposable gloves may be desirable, and a disinfecting hand cleaner should be used as soon as possible after collecting samples. Equipment exposed to potentially contaminated water should be washed in soap and water and rinsed in clean water if possible.

Store Colilert® (media) according to directions and use before expiration date. Avoid prolonged exposure of media to direct sunlight. Discard media that have changed color, appearance, and/or texture (media are hygroscopic and will clump and darken if exposed to moisture)

In samples with excessive chlorine, a blue flash may be seen while adding Colilert or Colilert-18 media. If this occurs, consider sample invalid and discontinue testing.

MST samples containers must be kept clean and handled with disposable gloves when sampling.

3.3 Safety Precautions

Do not look directly at the Ultraviolet (UV) bulb during enumeration. Artificial UV sources can cause severe eye damage.

As discussed in Section 3.2, *E. coli* is a pathogen and indicator of other potential pathogens harmful to humans in water. Care must be taken when sampling potentially contaminated water and when handling activated media. See Job Hazard Analysis for sample collections.

Activated media are considered a biohazard and must be properly disposed of according to procedures outlined in the most recent approved Chemical Hygiene Plan (CHP; NMED/SWQB 2017 or current version).

4.0 Definitions

Deionized (DI) Water – Water that has been treated to remove nearly all dissolved substances.

Microbial Source Tracking (MST) - method for the identification of fecal pollution sources in environmental waters that relies on genetic markers unique to bacteria associated with certain hosts.

Program Manager – An individual within the SWQB that manages a program such as the Watershed Protection Section (WPS), the Point Source Regulation Section (PSRS) or the Monitoring, Assessment and Standards Section (MASS).

Project Manager(s) – An individual responsible for a specific project identified in a field sampling plan or Sampling Analysis Plan. This individual, in most cases, holds a different title within the organization.

Quality Assurance Officer (QAO) – An individual within the MASS that is responsible for overseeing the development and implementation of all quality assurance procedures and processes within the SWQB including those projects that receive support or funding from the SWQB. The QAO is also responsible for validating and verifying data sets for potential use in assessment of surface waters.

Quality Assurance Project Plan (QAPP) – A formal planning document for environmental data collection activities that describes the data collection procedures and the necessary quality assurance and quality

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control activities that must be implemented to ensure that the results are sufficient and adequate to satisfy the stated performance criteria.

Run/Sampling Run – A period of time used to represent and define the most common collecting period or grouping of sampling activities that are indicative of SWQB sampling operations. Typically, most samples are collected during multi-day collection events that depart and return to the office in a given week (M-F). For the purposes of this SOP, qualification resulting from blanks associated with sample collection during a run is also limited to a single week, which may include single or multi-day collection activities. Also, blanks associated with a run are assumed to collectively represent a grouping of samples whereby the staff, equipment, vehicle, reagents, preservation, and storage remain constant. When multiple single day trips are planned within a given week that maintain constant variables as described above, the single day trips may be considered collectively as a run, provided that the number of blank QC samples collected represents at least 10% of ambient samples collected, preferably at a frequency of one blank sample per ten ambient samples to allow for adequate QC blank distribution.

Subject Matter Expert (SME) – A person who is familiar with the purpose and procedure for accomplishing a task. The SME may be the same individual as the Program or Project Manager.

Surface Water Quality Information Database (SQUID) – The SWQB database for storing, retrieving and reporting environmental results which include laboratory results, field observations, biologic assemblage data, long-term datasets (LTD), summary data, and stream habitat/geomorphic data.

5.0 Equipment and Supplies

5.1 Equipment and Tools

There are two primary sets of IDEXX equipment available through the SWQB laboratory: a lab kit and a field kit.

Lab Kit:

- 110V Incubator
- Fluorescent UV lamp (365nm to 366nm wavelength)
- IDEXX UV Viewing Cabinet
- IDEXX Quanti-Tray® sealer
- IDEXX Quanti-Tray®/2000 rubber insert
- Pipette (measurement calibration error for a given manufacturer’s lot must not exceed 2.5%. Use pipets with distinctly marked graduations and unbroken tips. Bacteriological-transfer pipets or pipets conforming to American Public Health Association (APHA) standards may be used)
- Graduated cylinder (meeting ASTM International standards (D-86 and D-216) and with accuracy limits established by NIST)

Field Kit:

- Portable Incubator with DC power plug for vehicle operation
- IDEXX 6-watt fluorescent UV lamp (365nm to 366nm wavelength)
- IDEXX UV Viewing Cabinet
- IDEXX Quanti-Tray® sealer
- IDEXX Quanti-Tray®/2000 rubber insert

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- AC/DC converter to power the portable incubator via a typical 110V outlet
- DC/AC 2500-watt inverter and: +/- alligator clips to allow connection of sealer to vehicle alternator
- DC/DC adapter allowing vehicle power port conversion to +/- alligator clips for deep cycle battery power
- Deep cycle battery(s) if overnighting without an AC power source
- Extension cord
- Pipette (measurement calibration error for a given manufacturer's lot must not exceed 2.5%. Use pipets with distinctly marked graduations and unbroken tips. Bacteriological-transfer pipets or pipets conforming to American Public Health Association (APHA) standards may be used)
- Graduated cylinder (meeting ASTM International standards (D-86 and D-216) and with accuracy limits established by NIST)

Additionally, there is an extra power converter, fluorescent light and portable field incubator in the SWQB laboratory. If it becomes necessary for two teams to culture bacteria in the field, the lab sealer may be checked out. Prior to doing so, a notice must be sent to the SWQB ensuring that the lab sealer is not scheduled for use during that time.

5.2 Supplies

Materials for Samples Processed by SWQB:

- IDEXX Colilert® Reagent "Snap Packs" for 24-28 hour incubation
- IDEXX Colilert®-18 Reagent "Snap Packs" for 18-22 hour incubation
- IDEXX Quanti-Tray®/2000 bacterial enumeration trays
- IDEXX Sample 120mL Bottles containing sodium thiosulfate
- Colilert® and Colilert®-18 Quanti-Tray®/2000 Comparator
- Clean Deionized (DI) water for blank collection or dilution

Materials for Samples processed by SLD:

- 125 ml, sterile polypropylene sample bottles (available from SLD)
Note: SLD does not accept samples in IDEXX bottles
- "Do not tamper" seals for lids (available from SLD)
- Submittal form (download from <http://nmhealth.org/publication/view/form/1498/>)
- Clean DI water for blank collection

Materials for MST Samples:

- 1L polyethylene or polypropylene bottle with screw cap

The Environmental Microbiology section at SLD should be consulted (505-383-9122) at least 2 weeks in advance of the proposed sampling date to assure availability of media and incubator space.

6.0 Process Description

Collect both routine and quality control (blank) samples for total coliform and *E. coli* sample analysis. Collect one set of quality control samples with each group of samples collected on a sampling run (with the exception of MST samples). Ten percent of the samples should be quality control samples. Prepare

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the field blank using Deionized (DI) water and process quality control samples the same as a routine sample.

For the IDEXX method, prior to going in the field, ensure that you have the “Bacteria Record Sheet” in hard copy or electronic format (within SLD Submittal Forms) and use this form to record all required information for each sample.

For total coliform and *E. coli* samples submittal to SLD, contact SLD in advance of delivery of the samples to verify that the samples can be processed within the required time. (**NOTE:** Maximum transport time is **6 hrs.** from time of collection until time of delivery at SLD per their requirements – see below holding time discussion in Section 6.1). Be sure to obtain and follow SLD specific instructions when making sample arrangements and obtaining containers from SLD.

For MST samples, ensure that you have scheduled the analysis with a contract laboratory and have arranged shipping, preferably within 24 hours of sample collection or as soon as possible to ensure adequate time for laboratory sample processing. The hold time for MST samples varies by laboratory and analysis. It is recommended that sample container, preservation method and hold time be verified each field season prior to sampling, when a new laboratory is utilized for analysis or if personnel are unexperienced with NMED SWQB MST sampling protocols. Currently, the laboratory used by the SWQB will flag samples that arrive 48 hours after sample collection (see Section 8.0 Quality Control and Quality Assurance of this SOP for additional information).

6.1 Total Coliform and *E. coli* Sample Collection, Storage, and Hold Times

Use IDEXX sample bottles (120-mL containers with sodium thiosulfate) or sterile polypropylene sample bottles provided by SLD. Do not rinse sample bottles before use and do not remove the caps or shrink wrap until immediately before sampling. Collect samples directly into the sample bottles whenever possible. If it is necessary to transfer to the sample bottle from another vessel, note this in the comment field when the sampling event is entered into SQUID.

Verify that the bottle is labeled with the sample ID on the bottle, then remove and dispose of the shrink band or sealing tape. In a stream, it is preferable to sample where the stream is flowing, well mixed and more than 6 inches deep. Stand downstream of the sampling bottle to avoid getting streambed sediment in the sample. In a lake or reservoir, collect samples from the water surface off the side of a boat at the sampling station or from the water’s edge more than 6 inches deep when collecting samples from shore.

Wear disposable gloves if you are sampling effluent water or are working downstream of a wastewater treatment plant. Uncap the sample bottle, while holding the cap in one hand and the bottle in the other, submerge the bottle in as upright a position as possible to retain the sodium thiosulfate chlorine neutralizer. Collect a sample from the water column, minimizing the amount of surface material entering the bottle. Attempt to fill the bottle just to the 100-mL line for IDEXX bottles or to the shoulder line for SLD bottles. If the bottle is filled above the 100-mL line or shoulder line, immediately decant excess water. Carefully replace the cap.

After collecting the sample, ice or refrigerate to less than 10°C. Use insulated containers to assure proper maintenance of storage temperature. Ensure that sample vessels are not totally immersed in water during transit. Place samples in a plastic bag and put samples on top of other iced samples.

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To ensure that the most accurate data are generated, *E. coli* samples collected from surface waters should always be analyzed as soon as possible (Thapa et al. 2020). As a standard practice, SWQB processes and incubates IDEXX samples for routine monitoring and assessment purposes within 8 hours of sample collection whenever possible. In the rare event that it is not possible to process within 8 hours of sample collection, hold times of up to 24 hours are permissible for routine monitoring and assessment purposes assuming a sample storage temperature remains below 10 degrees C (APHA 2017, Thapa et al. 2020). Submit SLD samples within 6 hours of sample collection per their requirements.

Note: Complaint/Spill response samples and discharge permit monitoring samples used for state regulatory purposes must be processed and incubated within 8 hours of sample collection (APHA 2017).

6.2 Total Coliform and *E.coli* Sampling Methods for Samples Requiring Dilution

New Mexico's water quality standards contain a single sample *E. coli* criterion of 2507 cfu/100mL or MPN/100mL for the Secondary Contact designated use. Undiluted, the maximum reporting level of the IDEXX Colilert and Colilert-18 methods is 2419.6 MPN/100mL. It may be necessary to dilute surface water samples taken in assessment units with a Secondary Contact designated use or in other cases where a higher maximum reporting level is desired. Dilution of water samples with DI water increases both the minimum and maximum reporting levels of the IDEXX methods.

Use the following procedure for dilution:

- Using a clean pipet or graduated cylinder, extract the desired amount of clean DI water and place into the 120mL IDEXX sample container. For this example, 5 mLs of DI is used. **Note:** A dilution amount of 5mL is all that is needed to detect above NM's Secondary Contact designated use single sample criterion.
- Take a clean graduated cylinder and rinse it thoroughly twice with the water to be sampled.
- Take a clean container (>100mL size) and rinse it thoroughly twice with the water to be sampled.
- Fill the clean container with sample water.
- Slowly pour in sample water from the container into the graduated cylinder until you reach the desired sample water volume (95mL in this example).
- Pour the sample water (95mL in this example) from the graduated cylinder into the Idexx sample container with aliquot of DI water (5mL in this example) for a total sample size of 100 mL.
- Ice or refrigerate to less than 10°C and process within 8 hours if sampling for a complaint/spill response or discharge monitoring used for state regulatory purposes (see Section 6.1 regarding hold times up to 24 hours).
- Record or enter the amount of undiluted sample on the Bacteria Record Sheet.

6.3 Processing Total Coliform and *E. coli* Samples using IDEXX Media for Incubation and Enumeration in the SWQB Laboratory

Start incubation within 8 hours of sample collection. **Note:** see Section 6.1 for detailed hold time discussion. Use the following procedure to process the samples in the SWQB Laboratory:

- Remove the samples from the cooler approximately 30 minutes prior to arriving at the processing site and allow them to warm to ambient temperature. This allows for the reagent to easily dissolve.

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- Switch on the Quanti-Tray® Sealer and allow it to warm up until the green light on the cover comes on.
- Fill out a Bacteria Record Sheet and use this form to record all required information (e.g., media, incubator temperature, time and date of incubation) for each sample.
- As appropriate based on read-time, select either Colilert®-18 (18-22 hour incubation) or Colilert® (24 -28 hour incubation) reagent.
- Add one reagent packet directly to each sample bottle then cap the bottle and shake vigorously until reagent is dissolved. Tests should be placed in the incubator within 30 min after reagent is added to sample.
- Label the Quanti-Tray®2000 with the RID number using a marker or extra RID sticker. Avoid using pens or pencils as they can poke holes in the Quanti-Tray®2000.
- Gently pull foil tab on a Quanti-Tray®2000 incubation tray to separate foil from tray, being careful not to touch the inside of either foil or tray. Pour the solution into the tray while holding it vertically and avoiding contact with foil tab. Gently tap the small wells 2 to 3 times to release any air bubbles that may be trapped. Allow foam to settle, although some foam is acceptable.
- Place the tray into the rubber frame and insert the frame and tray into the bay in the front of the sealer.
- Carefully ease the frame and tray forward until the sealer activates and feeds the tray automatically. The sealer may occasionally jam while processing the tray. If this occurs, press the reverse button on the cover and the track will reverse, depositing the frame on the dock. Wait until the green light activates again and repeat the loading process. The tray will be deposited on the counter behind the sealer.
- Place the sealed tray into the 35°C incubator and incubate 18-22 hrs. for Colilert®-18 reagent or 24-28 hrs. for Colilert® reagent.
- Record initial incubator temperature and incubation date and time on the Bacteria Record Sheet.

Use the following procedure to enumerate the samples in the SWQB Laboratory:

- After the appropriate incubation period, remove the sealed tray and record the final incubator temperature and removal time and date on the Bacteria Record Sheet.
- Enumerate total coliform bacteria by counting the number of large and small cells that turn yellow after incubation, the large cell at the top of the tray is counted as the 49th large cell. Record observations on the Bacteria Record Sheet. Enumerate *E. coli* by counting the number of large and small cells that fluoresce under illumination with the IDEXX 6-watt fluorescent UV lamp. The large cell at the top of the tray is counted as the 49th large cell. Record observations on the Bacteria Record Sheet.
- For both total coliform and *E. coli*, the Colilert® and Colilert®-18 Quanti-Tray®/2000 Comparator should be used to verify positive or negative cells.
- If not already complete, enter the sample date, method, sample ID (RID#), and the number of positive large and small cells counted for total coliform and *E. coli* into the Bacteria Record Sheet found within the SLD Submittal Forms (see the SWQB SOP 8.2 on the website for the most recent version). Press “Calculate MPN” to calculate the mpn/100 ml and the upper and lower boundaries of the 95% confidence interval. Qualifiers for incubation time, hold time, incubation temperature, and reporting level are generated by the calculation routine. Verify that data entry is correct and that calculated qualifiers are appropriate, including rejected trays.
- Confirm that there are no data QA issues and then press “Format Upload” to create the data file and then press the “Save and Export” button to save file for SQUID database upload. Additional instructions and details are provided on the Bacteria Record Sheet and SLD Submittal Form.

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6.4 Processing Total Coliform and *E. coli* Samples using IDEXX Media for Incubation and Enumeration in the Field

A field kit has been assembled for IDEXX sample processing in the field. Procedures are the same as described in Section 6.1-6.3 of this SOP, however a 12-volt (DC) incubator, adaptors and voltage converters are used to operate the equipment (e.g., Quanti-Tray® Sealer) using the electrical system of a vehicle. There is a checkout sheet for the field kit in the SWQB public folders: [SWQB PUBLIC\Sonde&Equipment Checkout](#). Use this sheet when taking the kit from the building.

Before leaving on a sampling run, it is advisable to test all field kit components to ensure that they are all present and working.

Use the portable DC incubators for field incubation. These are supplied with a power cord that allow them to be plugged into a vehicle's power outlet. However, they draw sufficient amperage and can drain the vehicle battery if the vehicle is not running for long periods of time. If the vehicle will not be running for an hour or more it is recommended to power the incubator from a deep cycle battery. A fully charged deep cycle battery in good condition can run the incubator up to 16 to 24 hours (2 nights). There is also an AC/DC converter that converts 110V AC to 12V DC allowing the incubator to be powered by a standard home 3-prong electric outlet for use in a hotel room, campsite, or field office. The power cord for the Thermotote® model can be inserted two ways: one orientation will heat the incubator (red lamp lights) and the other will cool it (green lamp lights). **Before processing samples for incubation ensure the incubator has heated to at least the minimum incubation temperature. It may be necessary to turn the incubator on several hours before processing to ensure the incubator has warmed up.**

If it is necessary to seal Quanti-Trays in the field, the IDEXX sealer may be powered by the vehicle alternator. To operate the 110V sealer, power must be converted from the 12V supplied by the vehicle alternator, and the vehicle must be running to supply sufficient amperage. A DC/AC 800-watt inverter is used for this power conversion. Use the following procedure to use the 110V sealer on the 12V system:

- Clamp the alligator clips on the leads to the converter to the respective terminals on the vehicle battery.
- Plug the sealer into the converter.
- Start the vehicle.
- Turn the inverter on.
- Turn the Quanti-Tray® Sealer on and allow it to warm up until the green light on the cover comes on. It may be necessary to place the sealer inside the vehicle, close vehicle doors, and turn the heat on during cold conditions.

Follow incubation procedures described in 6.3 Processing Total Coliform and *E. coli* Samples using IDEXX Media for Incubation and Enumeration in the SWQB Laboratory.

It may be necessary to enumerate Quanti-Trays in the field. The biggest challenge to this task is finding a dark place to discern which cells fluoresce. Parking the vehicle in the shade and placing sample on the floor often provides enough darkness to see fluorescing cells under UV illumination with the UV light. Cardboard boxes, jackets, WWTP laboratories and SWQB field offices are often used for this purpose.

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Follow enumeration procedures described in 6.3 Processing Total Coliform and *E. coli* Samples using IDEXX Media for Incubation and Enumeration in the SWQB Laboratory.

6.5 Disposal of Quality Trays

Incubated Quanti-Trays are considered microbiological waste and must be disposed of accordingly. Trays collected in the field must be stored in a red “biohazard” bag and returned to the SWQB laboratory for storage. A red biohazard storage container is kept in the SWQB laboratory walk-in cooler for staging of all enumerated Quanti-Trays, and is periodically transferred to SLD for disposal. Consult the SWQB Health and Safety Officer for more information on handling and storage of enumerated Quanti-Trays as necessary.

6.6 MST Sampling Procedure

Wearing latex or nitrile gloves, fill and rinse a 1L poly bottle with sample water twice, then fill the bottle with at least 1L of sample water. Take care to avoid contaminating the inside of the sample bottle with skin, dirt or other debris. Screw on the cap and store on ice or refrigerate at less than 6°C. Ship the MST samples to the contract laboratory within 24 hours using overnight shipping. Dependent on sample degradation and laboratory procedure, MST sample hold time could be up to 7 days after sample collection (Saito and Doi 2021) if sample temperature remains at less than 6°C and passes laboratory quality assurance protocols. More information is detailed in the Quality Control and Quality Assurance Section of this SOP.

7.0 Data and Records Management

Data recorded on hard copy field forms (Bacteria Record Sheets) need to be transferred to the electronic version of the Bacteria Record Sheet found within the SLD Submittal Forms workbook used to format and upload field data from sampling runs to SQUID. MST samples are documented on the Stream and River Field Data Form or the Lake and Reservoir Field Data Form. Data recorded on hard copy field forms are kept in the project file.

7.1 Uploading Total Coliform and *E. coli* Data to SQUID

1. To upload bacteriological sampling data and results into SQUID, utilize the Bacteria Record Sheet found within SLD Submittal Forms with the stream or lake sampling/water chemistry information populated.
2. Ensure that all data are complete in the Bacteria Record Sheet, including the unit where bacteria was incubated, RIDs, station IDs, sampling/incubation dates and times, Colilert used, undiluted sample volume, count date/times, start/count temperatures, and positive counts.
3. Press “Calculate MPN and 95%.” MPNs, upper/lower confidence intervals, and SWQB qualifiers should appear.
4. Ensure that qualifiers are correct. Ensure no transcription errors occurred to falsely qualify data.
5. Press “Format Upload” Review Bacteria File Upload worksheet to ensure file contents and formatting are correct.
6. Return to the Bacteria Record Sheet and press “Save and Export.” A message will appear: “Export Bacteria Upload File?”. Select “yes.”

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7. A bacteria upload file will be automatically saved in the user's C drive, under the folder "Field_Sheets." Note: the user must have a folder titled "Field_Sheets" in user's C drive for the "Save and Export" button to work properly.
8. The file will be named "Bacteria_UL_date." Copy and save the upload to the "Bacteria" folder in the project file.
9. In SQUID, select the *Data Management* tab. Select *Imports* and then select *SLD Results* from the sub-menu.
10. In the *Import SLD Data* page, select "choose file" and select the bacteria upload file. Select the SQUID "upload file" button.
11. Verify that bacteria results appear in the database.

7.2 Creating and Uploading MST Sampling Event and Results into SQUID

Results for MST are reported by a contract laboratory in a similar fashion as chemical data analyzed by SLD, therefore the SWQB utilizes the procedure for Uploading Chemical Sampling Event Data Manually into SQUID described in SWQB SOP 8.2 for Chemical Sampling in Lotic Environments. The referenced procedure should be used by SWQB staff when uploading MST samples events into SQUID.

The QAO uploads MST results within 7 days of receiving data from contract laboratory. See QAO for additional details regarding MST result upload procedure.

8.0 Quality Control and Quality Assurance

If total coliform and *E. coli* samples are analyzed by SLD, the Laboratory should provide information to verify that the incubation temperature was maintained within the method-specified range, that the incubation began within 2 hours after sample receipt, and that the incubation time was within that required by the method.

Annually, or preferably semiannually, verify the accuracy of all working temperature-sensing devices (e.g., incubator) **at the use temperature(s)**. To do this, compare device's measurements to those of a certified NIST temperature-sensing device or one traceable to NIST and conforming to NIST specifications.

If total coliform and *E. coli* samples were processed using SWQB equipment, the following sample rejection rules apply:

If both the temperature at the initiation and conclusion of the incubation were within $35 \pm 0.5^\circ\text{C}$, the data is not flagged (i.e. no qualifier code) . If either temperature is less than 34.5°C , the data is discarded. If either temperature is between 35.5 and 38°C , the data is flagged and may only be used as supporting evidence for assessments (qualifier code = "Ea"). If either temperature is greater than 38°C , the data is rejected (qualifier code = "Er").

The sealed trays should be read within the period indicated by the reagent. If trays are read before the nominal incubation time or more than 4 hours after, the data are rejected unless read more than 4 hours after nominal incubation time and results are negative, in which case an "S2" qualifier is applied with a comment on incubation time. If samples are not processed within 8 hours of sample collection a "K1" qualifier code is applied with a comment stating results may not be used for compliance. Any

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samples not processed within 24 hours of sample collection will be rejected and a R1 validation code will be applied.

Any person processing samples need to ensure the proper SWQB validation codes are applied along with the associated WQX equivalent validation code, prior to uploading to SQUID. See Attachment B1-B3 of the SWQB SOP 15.0 Verification and Validation for more information.

If a blank shows a value greater than the blank validation criteria value, then all of the results since the last in-range sample are rejected. This is summarized below:

	Validation Criteria	Validation Codes and Action	
Undiluted Blank Sample	<1 mpn/100 mL	<1 mpn/100 mL, no code	≥1 mpn/100mL RB1, reject results
Diluted Blank Sample	<(1*100/undiluted volume) mpn/100mL	<(1*100/undiluted volume) mpn/100mL, no code	≥ (1*100/undiluted volume) mpn/100mL, RB1, reject results

The MST samples should be shipped to the contracted lab as soon as possible. In most cases, shipping MST samples overnight within 24 hours should ensure laboratory receipt within 48 hours of sample collection. The current contract laboratory quality assurance procedures for MST examination by quantitative polymerase chain reaction will alert the QAO through email for samples that arrive at 6°C or greater and/or the samples were not received within 48 hours of sample collection.

Although it is recommended to have the samples shipped and received by the laboratory within 48 hours of sample collection, literature suggest that MST sampled may still be viable if samples are received by laboratory within 7 days of sample collection and were maintained at a temperature of less than 6°C (Saito and Doi 2021). The contract laboratory will analyze MST samples at the discretion of the SWQB when hold time and preservation requirements are not met. When the temperature and/or 48 hour hold time are exceeded, SWQB staff will ensure MST results are flagged with the SWQB validation code of “K1”. MST data results will be rejected with a “R1” validation code if samples are not extracted by laboratory within 7 days of sample collection. The verification and validation procedure for MST samples and results should follow Chemical Data Verification and Validation detailed in SOP 15.0. **Note:** MST sampling does not include field blanks. Any SWQB validation code applied should include the associated WQX equivalent validation code prior to the completion of the verification and validation of MST samples.

Before use of MST results by the SWQB in decision making (e.g., Best Management Practice (BMPs) implementation, Total Maximum Daily Loads (TMDLs) development, etc.), the SWQB QAO should be consulted to ensure the proper quality assurance protocols were employed so that the decisions made based on the data are defensible for projects-specific objects and goals.

The SWQB controls the quality of the bacteriological sampling and analysis by using standardized methods that are documented in this SOP. All personnel who collect or analyze bacteriological samples must be familiar with these protocols, sign the acknowledgment form associated with this specific SOP and collect and analyze data in accordance with the procedures as they are defined in this SOP. In addition to standardized methods, proper training of field personnel represents a critical aspect of meeting the data quality objectives in order to fulfill the goals of the SWQB’s QAPP (NMED/SWQB, 2021). If, at any time, the QAO determines this process is not being adhered to, the QAO has the

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authority to cease activities specific to this SOP with prior support and approval by the SWQB Bureau Chief and MASS Program Manager, until such a time that the issue can be resolved.

For more information regarding quality assurance procedures contact the NMED SWQB QAO <https://www.web-t.env.nm.gov/surface-water-quality/>.

9.0 Related Forms

Bacteria Record Sheet found within the SLD Submittal Forms – located on the SWQB SOP webpage (<https://www.env.nm.gov/surface-water-quality/sop/>) SOP 8.2.

10.0 Revision History

Revision 0. March 21, 2011. Original.

Jodey Kougioulis, QAO; James Hogan, SME/Program Manager

Revision 1. April 3, 2013. No significant changes.

Gregory Huey, SME; Jodey Kougioulis, QAO; Shelly Lemon, Program Manager

Revision 2. April 20, 2015. No significant changes. Made QA frequency consistent with SWQB QA policies.

Gregory Huey, SME; Jodey Kougioulis, QAO; James Hogan, Acting Program Manger

Revision 3. April 20, 2016. Updated data processing and upload instructions. Added QC instructions for positive and negative control. Major updates and structural organization to entire SOP.

Shelly Lemon, Acting QAO; Chuck Dentino, SME; Shelly Lemon, Program Manager

Revision 4. November 17, 2020. Added dilution procedures, increased hold time for non-regulatory analysis to 24 hours, updated procedure, upload instructions, and updated field/upload form.

Miguel Montoya, QAO; Charles Dentino, SME; Kris Barrios, MASS Program Manager

Revision 5. October 5, 2022. Added MST sampling information, added graduated cylinder for dilution.

Miguel Montoya, QAO; Charles Dentino, SME; Heidi Henderson, Acting MASS Program Manager

Revision 6. March 14, 2023. Clarified SWQB monitoring and assessment standard practice of 8 hour *E. coli* hold time (non-regularly 24 hour hold time for rare circumstances). Added additional references and qualifier codes. Added MST sample hold time clarifications.

Miguel Montoya, QAO; Elizabeth Stuffings, SME; Lynette Guevara, MASS Program Manager

11.0 References

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